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Radiological and histological assessment of a novel interlocking three-dimensional miniplate for mandibular angle fractures: an animal study

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ABSTRACT

BACKGROUND Open reduction and internal fixation are considered the gold standard for managing mandibular fractures, as they effectively restore anatomical structure and functionality. Three-dimensional (3D) miniplates were developed to obtain a geometrically stable structure. However, the rigid structure of 3D miniplate may cause screw holes to be located within the fracture lines or directly above important anatomical structures. Hence, we have developed customizable interlocking 3D miniplates that allow for modifications in the configuration of the horizontal miniplates and vertical cross-struts to meet specific requirements.

METHODS This study included 24 goats divided into 2 groups: interlocking 3D miniplate and standard plate. Each group was further divided into subgroups sacrificed on Days 5, 28, and 56. Radiological assessments were performed to evaluate bone, muscle, peri-implant bone, and peri-implant muscle density. Histological assessments were performed using Masson's trichome (MT) for bone healing evaluation and hematoxylin and eosin (H&E) staining for biocompatibility.

RESULTS Radiological assessment indicated an increase in density across all measured parameters from Day 5 to 56. Histological evaluation from MT staining showed a significantly higher fibrocartilage in the interlocking 3D miniplate group (6.109%, IQR 1.453–10.828) compared to standard plate (1.311%, IQR 0.636–2.553; $p = 0.001$). Furthermore, H&E staining showed significantly higher inflammatory cell counts in the standard group on Days 28 and 56 compared to the interlocking 3D miniplate group ($p = 0.027$).

CONCLUSIONS This study concluded that the interlocking 3D miniplate showed better bone healing properties and biocompatibility than the standard plate.

KEYWORDS animals, bone plates, laboratory, mandibular fracture

Mandibular fractures pose significant challenges in achieving accurate anatomical restoration. The primary goal of treating these fractures is to restore both the structural integrity and functionality of the mandible. Open reduction and internal fixation are commonly employed to achieve this objective. Despite advances in treatment methods, the optimal approach for ensuring stable fixation remains debated.[1] We hypothesize that a novel interlocking three-dimensional (3D) miniplate design could offer superior stability in mandibular angle fractures than existing fixation techniques.

The development of internal fixation devices for mandibular fractures has evolved since Michelet first introduced them in 1973.[2] Champy later proposed a method for treating mandibular angle fractures using a single miniplate placed along either the oblique line or the superior border of the mandible.[3] This technique reduces skin incisions, minimizes damage to the inferior alveolar and facial nerves, and shortens surgical time. However, several studies have shown that Champy's method often needs more stability to properly align fracture fragments.[3]

Some studies have suggested using two miniplates placed in the tension and compression zones to address issues such as fracture segment separation and rotation. This approach allows for better anatomical repositioning of the fracture segments, overcoming the limitations of earlier techniques.[4] However, follow-up studies have yielded mixed results, with reports of improved stability, no significant differences, and even high complication rates.[5] To address these challenges, Farmand and Dupoirieux developed 3D miniplates that incorporate two plates connected by vertical cross-struts based on the stable geometry of a quadrangle structure.[6]

Current 3D miniplate designs, however, need to be more adaptable to support complex fracture patterns.[7] Existing designs may make screw placement difficult, as screw holes can fall along the fracture lines or over critical anatomical structures, such as dental roots and nerves. In response, this study presents a new 3D miniplate design that can be adjusted during surgery to overcome these challenges. We have developed interlocking 3D miniplates that can be customized by modifying the arrangement of the horizontal plates and vertical cross-struts to meet specific requirements. Our previous research demonstrated enhanced stability in the fixation of mandibular angle fractures compared to conventional miniplates through biomechanical testing.[8]

Ensuring the safety and efficacy of medical devices involves several key stages, including material testing, *in vitro* screening, and confirmatory *in vivo* testing. Animal studies play a crucial role in evaluating the safety and effectiveness of new devices, providing valuable data on biocompatibility and bone-healing properties.[9] Therefore, this study aimed to assess the biocompatibility and bone-healing properties of the novel interlocking 3D miniplates through radiological and histological evaluations in animal models.

METHODS

This experimental animal study compared the biocompatibility and bone-healing properties of a novel interlocking 3D miniplate with those of a standard to treat mandibular angle fractures in goats (*Capra hircus*). This study was approved by the Animal Ethics Committee, School of Veterinary Medicine and Biomedical Sciences, IPB University (Number: 017/KEH/SKE/II/2023). The study included healthy adult male goats aged 24–36 months, weighing 20–30 kg, with cortical bone density within two standard deviations of the mean. The study was conducted at the Veterinary Hospital, Divisions of Pathology, Surgery and Radiology, IPB University.

A total of 24 goats were randomly assigned to two groups: the interlocking 3D miniplate group and the standard plate group. Based on Federer's formula, each group was further subdivided with sacrifices scheduled 5, 28, and 56 days post-surgery, resulting in four goats per subgroup. The goats were acclimatized to their environment for 1 week prior to the procedure. They received antifungal and anti-scabies treatments during this period and were tagged for identification. The animals were housed in groups within identical care rooms to control temperature and humidity variations. The rooms were maintained with a regulated light–dark cycle, and the goats had unrestricted access to food and water.

Surgical procedure

All procedures were performed by the primary investigator. The goats were positioned in left lateral decubitus under general anesthesia. A 5 cm transverse incision was made behind the second molar on the right mandible, cutting through the skin and subcutaneous tissue with a no. 15 scalpel blades. The incision was extended through the muscle layer, and bleeding was controlled using electrocautery. The periosteum was incised and elevated over a 5 cm area at the mandible's angle, where osteotomy and fixation were performed. A controlled fracture was created using a reciprocating saw (OsteoPower™, USA), generating a fracture line from the last molar to the inferior angle, ensuring that both the outer and inner cortices were cut at the same level.

An interlocking 3D miniplate (Indonesia) or a standard plate (USA) was applied according to the assigned group. The subcutaneous tissue and periosteum were closed with interrupted absorbable 2.0 sutures. Postoperative care included daily oral antibiotics for 6 days, daily bandage changes, and wound care. Sutures were removed after 1 week if no signs of infection were present. After surgery, the goats were able to chew and consume grass.

Radiological assessment

Radiological evaluations were conducted immediately after surgery to confirm proper reduction as well as accurate plate and screw placement. Subsequent evaluations were conducted on postoperative Days 5, 28, and 56. Digital X-ray images were captured in both ventrodorsal and laterolateral views using a VR 1020 digital X-ray scanner (MA Medical, Japan). The images were analyzed with ImageJ software (National Institutes of Health, USA). For each region of interest (ROI) (bone, muscle, peri-implant bone, and peri-implant muscle density), measurements were taken at three points and averaged to represent the area.

Biopsy and histological assessment

Biopsy specimens were collected from the fracture site, including the bone directly beneath the plate and extending 1 cm beyond the plate on both sides. Surrounding soft tissues, such as muscle, fibrous tissue, and granulation tissue, were also removed.

Hematoxylin and eosin (H&E) staining was used to assess biocompatibility by counting the number of inflammatory cells. Masson's trichrome (MT) staining was employed to evaluate bone healing, specifically assessing the presence of fibrocartilage, woven bone, lamellar bone, and mature bone. This analysis was performed at 10× magnification across five fields of view, with cell counts expressed as a percentage of the ROI using ImageJ software.

Statistical analysis

Data were organized and coded in Microsoft Excel, then analyzed using the SPSS software version 27 (IBM Corp., USA). Statistical comparisons were made using the independent *t*-test, with the Mann–Whitney *U* test applied to data exhibiting non-normal distributions.

RESULTS

Radiological assessment

Both treatment groups exhibited an increase in bone and muscle densities throughout the study period. Both groups also demonstrated notable increases in the densities of bone, muscle, peri-implant bone, and peri-implant muscle from Day 5 to 56. However, when comparing bone, muscle, and peri-implant muscle densities between the two

Table 1. Comparison of bone, muscle, peri-implant muscle, and peri-implant bone densities in the interlocking 3D miniplate and standard plate groups

Time	Treatment	Variables			
		Bone density (a.u)	Muscle density (a.u)	Peri-implant muscle density (a.u)	Peri-implant bone density (a.u)
Postoperative (n = 12)	Interlocking 3D miniplate, mean (SD)	30.56 (2.25)	17.39 (1.06)	146.45 (27.57)	189.09 (6.28)
	Standard plate, mean (SD)	29.58 (2.39)	17.04 (2.35)	137.45 (24.19)	184.00 (4.99)
	*p**	0.311	0.645	0.43	0.43
Day 5 (n = 4)	Interlocking 3D miniplate, mean (SD)	31.43 (2.86)	18.78 (1.46)	134.00 (2.16)	185.00 (5.48)
	Standard plate, mean (SD)	31.78 (1.66)	18.14 (2.23)	143.25 (14.57)	178.00 (5.66)
	*p**	0.837	0.65	0.26	0.126
Day 28 (n = 4)	Interlocking 3D miniplate, mean (SD)	32.77 (1.87)	18.92 (3.03)	135.75 (8.73)	190.25 (0.96)
	Standard plate, mean (SD)	32.93 (2.19)	18.94 (3.27)	131.75 (2.36)	185.50 (3.11)
	*p**	0.912	0.993	0.41	**0.027**
Day 56 (n = 4)	Interlocking 3D miniplate, mean (SD)	31.53 (5.93)	18.96 (1.63)	144.75 (1.71)	195.00 (3.83)
	Standard plate, mean (SD)	33.71 (1.23)	19.4 (2.69)	147.25 (23.20)	186.25 (9.18)
	*p**	0.499	0.793	0.84	0.129

3D=three-dimensional; a.u.=arbitrary unit; SD=standard deviation
*Independent *t*-test

Table 2. Comparison of bone healing components in MT staining between the interlocking 3D miniplate and standard plate groups

Time	Treatment	Fibrocartilage (%), median (range)	Woven bone (%), median (range)	Lamellar bone (%), median (range)	Mature bone (%), median (range)	Inflammatory cells (%)
Day 5	Interlocking 3D miniplate	6.11 (1.45–10.83)	0.23 (0–1.29)	5.17 (1.70–8.52)	8.82 (4.26–13.72)	7.06 (5.35–10.32)
	Standard plate	1.31 (0.64–2.55)	0 (0–0)	2.32 (0.01–12.68)	6.162 (0.10–15.84)	9.23 (7.28–10.41)
	*p**	**0.001**	0.383	0.820	0.265	0.121
Day 28	Interlocking 3D miniplate	25.60 (7.08)	0 (0–0)	4.35 (0.85–11.75)	8.602 (1.91–13.60)	1.73 (0–2.44)
	Standard plate	26.09 (18.23)	0 (0–0)	2.79 (0–6.65)	6.11 (0–12.69)	3.07 (0.39–4.54)
	p†	0.498	0.947	**0.038**	0.355	**0.033**
Day 56	Interlocking 3D miniplate	6.61 (0.01–27.74)	0 (0–0.47)	4.85 (1.09–9.69)	10.37 (5.03–57.37)	0 (0–1.43)
	Standard plate	14.11 (1.37–24.51)	0 (0–2.05)	7.08 (0–16.42)	15.07 (0–31.96)	1.86 (0–3.91)
	*p**	0.495	0.211	0.989	0.174	**0.023**

3D=three-dimensional; MT=Masson's trichrome
*Mann–Whitney *U* test; †independent *t*-test

groups, no significant differences were found at postoperative Days 5, 28, and 56 ($p>0.05$). In the interlocking 3D miniplate group, peri-implant bone density also increased from Day 5 to Day 56, similar to the trend seen in the standard plate group. Significant differences between the two groups were observed only on Day 28 ($p = 0.027$), as presented in Table 1.

Histopathological assessment

MT staining

On postoperative Day 5, the interlocking 3D miniplate group demonstrated greater levels of fibrocartilage, woven bone, lamellar bone, and mature bone than the standard plate group. Higher fibrocartilage formation was in the interlocking 3D miniplate group (Table 2).

By postoperative Day 28, no significant differences in fibrocartilage formation were observed between the two groups, nor was there any presence of woven bone. However, the percentage of lamellar bone was significantly higher in the interlocking 3D miniplate group. No significant difference in mature bone formation was observed between the two groups (Table 2).

By postoperative Day 56, no significant differences were noted in the levels of fibrocartilage, woven bone, lamellar bone, or mature bone between the interlocking 3D miniplate and standard plate groups, as seen in Table 2.

H&E staining

On postoperative Day 5, the interlocking 3D miniplate group showed a lower median number of inflammatory cells than the standard plate group, though this difference was not significant ($p = 0.121$).

On Days 28 and 56, the interlocking 3D miniplate group showed a lower median number of inflammatory cells. Both groups exhibited a decrease in the median number of inflammatory cells over time, with the differences between the groups at these time points being significant ($p<0.05$) (Table 2).

DISCUSSION

These studies provide valuable insights into the biocompatibility and bone-healing properties of newly designed devices. Goats were selected as the animal model for several reasons: their anatomical structure closely resembles that of humans, particularly the trabecular bone density of the alveolar bone.[10] Additionally, goats are readily available, have a bone remodeling rate similar to that of humans, and exhibit chewing movements that mirror human mastication, including lateral/transverse bending, parasagittal bending, reverse parasagittal bending, and torsion during chewing.[11] These characteristics make goats an ideal model for assessing the safety and efficacy of novel mandibular internal fixation devices intended for human use.[12]

Radiological evaluations were performed using X-ray imaging of the lateral and dorsoventral projections immediately after surgery, and on Days 5, 28, and 56 postoperatively. These time points were selected to capture key stages of bone healing. The results revealed no significant differences in bone density between the interlocking 3D miniplate and standard plate groups, although both groups showed an increasing trend in bone density. The initial increase in bone density observed on postoperative Day 5 in both groups is likely due to the early stages of bone healing.

Four weeks after surgery, granulation tissue had formed and transitioned into connective tissue, with increased vascular tissue emerging from the fracture edges. Angiogenesis, which is essential for bone healing, was evident as it facilitates the delivery of osteoclasts and osteoblasts to the fracture site. Our findings indicated increased bone healing activity at the fracture site, with higher bone density observed on Day 28. These results are consistent with the study by Al-Tairi et al,[13] who also reported sustained increases in bone density up to 6 months postoperatively, with no significant differences between the 3D miniplate and standard plate groups.

The peri-implant bone area is defined as the bone directly beneath the plate. This area was evaluated to assess bone reactions to the implant. Peri-implant bone density showed an increasing trend on postoperative Days 5, 28, and 56 for both groups, but there were no significant differences between the groups.

Important findings related to bone healing were noted in both groups. On postoperative Day 5, the interlocking 3D miniplate group exhibited a significantly higher percentage of fibrocartilage, indicating a more advanced stage of bone healing than that in the standard plate group. By Days 28 and 56, both groups were likely entering the remodeling phase, with increased lamellar bone and decreased woven bone. The interlocking 3D miniplate group showed superior bone healing early on, with significantly more fibrocartilage on Day 5 and more lamellar bone on Day 28 than the standard plate group. However, by Day 56, differences between the groups diminished, with no significant differences in woven and lamellar bone proportions. Additionally, mature bone levels peaked earlier in the interlocking 3D miniplate group (Day 28), while the standard plate group reached peak levels on Day 56. This suggests that the interlocking 3D miniplate accelerates early bone healing, although both plate types ultimately provided similar stability in the final phase of healing. These findings were supported by radiological assessments, which showed comparable increases in bone density for both plate types, with no significant differences. The superior bone healing in the interlocking 3D miniplate group is likely attributable to its ability to better stabilize fracture fragments, as demonstrated in previous biomechanical testing, which found minimal displacement with the interlocking design.[8]

Muscle density increased up to Day 56 in both groups, but no significant differences were observed. This may reflect ongoing muscle healing in response to the osteotomy and fixation procedures. Muscle density indicates the surrounding tissue's response to the injury caused by the surgery. The osteotomy and fixation initially affect the muscle tissue surrounding the fracture site, triggering an inflammatory response that likely leads to increased muscle density, particularly in the early stages. The increase in muscle density observed beyond Day 5 may also be due to the activity of scavenging cells during the bone-healing and remodeling phases.

The density of the peri-implant muscle area, which is located directly above the implant, was assessed to evaluate the reaction of the surrounding soft tissues to the implant. In this study, the density increased in both treatment groups up to Day 56, with no significant differences between the groups. This suggests that both implants triggered similar reactions in the surrounding bone and soft tissues.

H&E staining results aligned with the expected phases of bone healing. On Day 5, both treatment groups had higher median inflammatory cell counts than Days 28 and 56, reflecting the inflammatory phase of healing. The standard plate group showed a higher median number of inflammatory cells than the interlocking 3D miniplate group, although this difference was not significant.

By Day 28, both groups exhibited a reduction in median inflammatory cell counts, with the interlocking 3D miniplate group showing a significantly greater decrease, suggesting a more efficient resolution of the inflammatory phase. By Day 56, both groups showed the lowest median inflammatory cell counts, indicating the transition from the inflammatory phase to the remodeling phase of bone healing. The lower inflammatory cell count in the interlocking

3D miniplate group likely resulted from its ability to better stabilize the fracture fragments, as supported by prior biomechanical testing.[8] Since both miniplates used in this study were made from the same materials and produced by the same method, the observed differences in inflammatory response are unlikely to be related to the plate material itself. Additionally, previous toxicity evaluations have shown that the interlocking 3D miniplate has similar results to the commercially available Zimmer Biomet® miniplate, reinforcing the conclusion that the inflammation response is not material-dependent.

Understanding the dynamics of bone healing is critical when selecting the appropriate fixation device, as it depends on various clinical factors, including expected healing rates and postoperative mechanical stability. This study demonstrated that the interlocking 3D miniplate design offers superior bone healing and biocompatibility than a standard plate. Notably, the interlocking 3D miniplate group exhibited faster bone healing, with significant increases in fibrocartilage and lamellar bone at earlier time points. Although mature bone levels and overall bone density were similar between the groups by Day 56, biomechanical testing confirmed that the enhanced stability provided by the interlocking 3D miniplate contributed to improved healing and reduced inflammatory response. Both radiological and histological assessments showed a trend toward fewer inflammatory cells and reduced tissue reactions in the interlocking 3D miniplate group. In conclusion, this study suggests that the interlocking 3D miniplate offers superior bone-healing properties and biocompatibility than the standard plate. However, further studies on human subjects are necessary before clinical application can be considered.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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